Human sperm head vacuoles are physiological structures formed during the sperm development and maturation process

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Objective: To clarify whether human sperm vacuoles affected intracytoplasmic sperm injection (ICSI) success rates.
Design: Retrospective study.
Setting: A private infertility clinic.
Patient(s): Spermatozoa and spermatids were obtained from 11 normozoospermic, 10 oligozoospermic or asthenozoospermic, 4 obstructive azoospermic, and 3 nonobstructive azoospermic men.
Intervention(s): Differential interference contrast observation and intracytoplasmic injection of morphologically selected sperm.
Main Outcome Measure(s): Incidence, size, and position of vacuoles of sperm cells were recorded. Ability of fertilization and blastocyst development were compared between cells with and without vacuoles.
Result(s): More than 97.4% of ejaculated, 87.5% of epididymal, 87.5% of testicular spermatozoa, and more than 90.0% of Sc-Sd2 spermatids had vacuoles of various sizes. The incidence of vacuoles on ejaculated cells was significantly higher than that on the other types of cells, but there was no difference between sperm from normozoospermic men and those from the other donors. Removal of plasma membrane and/or acrosome did not affect the incidence of vacuoles. Although more than 60% of spermatozoa had small vacuoles in the acrosomal regions, 52.6% of Sb1-2 spermatids had large vacuoles. After injection of a motile spermatozoon with large and small vacuoles, 60.9% and 85.7% of metaphase II oocytes could be normally fertilized, respectively, and almost half of the zygotes developed to the blastocyst stage. When using sperm without vacuoles, the fertilization rate was 80.0%, but only 25% of them developed to the blastocyst stage.
Conclusion(s): Human sperm head vacuoles did not affect ICSI outcomes. (Fertil Steril® 2012;98:315–20. ©2012 by American Society for Reproductive Medicine.)
Key Words: Vacuole, spermatozoa, spermatid, spermiogenesis, epididymal maturation

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Along with a dramatic improvement in the quality of microscopes, intracytoplasmic sperm injection (ICSI) has become feasible at high optical resolution, and the significance of vacuoles of human sperm heads (1, 2) has been reevaluated. The vacuole is a concavity extending from the surface of the sperm head to the nucleus through the acrosome. According to Fawcett (3), the universal presence of vacuoles in the human sperm nucleus was documented in a report written by Eimer (4) more than a century ago. In the 1950s, transmission electron microscopy (TEM) of an ultrathin cross-section of the sperm head was carried out, which showed that a human sperm nucleus usually contained one or more vacuoles at different locations (5–7). On the basis of their universal presence in human sperm heads, most researchers concluded that intranuclear vacuoles should not be considered as degenerative structures with no physiological significance but instead should be regarded as a normal feature of the sperm head (3, 8–10). Meanwhile, after IVF techniques, including ICSI, were introduced in human infertility treatments, Mundy et al. (11) reported that sperm nuclei in subfertile men

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contained significantly more intranuclear vacuoles than did fertile controls. More recently, it has been reported that ICSI of sperm with vacuoles tended to result in decreased pregnancy rates (PR) and cause early miscarriage, and it has been proposed that vacuoles are not just a polymorphism but pose a risk for an abnormality that is accompanied by DNA injury (12–19).

To clarify these contradictory evaluations of the significance of human sperm vacuoles and establish whether this feature is physiological and normal, we have used the Normarski differential interference contrast (DIC) system to examine the incidence of vacuoles in male germ cells at various developmental and maturation stages (elongating and elongated spermatid, testicular, epididymal, and ejaculated spermatozoa) with or without initial demembranating treatments. Furthermore, we performed intracytoplasmic injection of morphologically selected sperm (IMSI) using motile normal-shaped sperm with or without vacuoles, and compared the results to our clinical data using conventional ICSI.

MATERIALS AND METHODS

Subjects and Ethical Aspects

This retrospective study was conducted with the informed consent of all participating patients. Round and elongating spermatid, testicular, epididymal, and ejaculated spermatozoa were obtained from 28 men (mean age, 37.7 $\pm$ 5.6 years; range, 31–50 years), including normozoospermic (11 men), oligozoospermic or asthenozoospermic (10 men), obstructive azoospermic (4 men), and nonobstructive azoospermic patients (3 men). Metaphase II oocytes were collected from 18 women (mean age, 34.6 $\pm$ 1.9 years; range, 28–37 years) undergoing controlled ovarian hyperstimulation (COH) according to the long GnRH agonist protocol (20). The Institutional Review Board of the Saint Mother Obstetrics and Gynecology Clinic approved the experimental use of such gametes.

Preparation of Ejaculated Spermatozoa

Semen samples were collected by masturbation and allowed to liquefy for 30 minutes at 37°C, followed by routine assessment of sperm characteristics.The patients were classified as giving oligozoospermia, asthenozoospermia, and normozoospermia according to the World Health Organization laboratory manual (21). These semen samples were centrifuged at 1,300 rpm for 8–10 minutes to remove the seminal plasma, and the spermatozoa were resuspended in homemade human tubal fluid (HTF) medium supplemented with 0.3% human serum albumin (HAS; Irvine Scientific) and kept at 37°C before observation for vacuoles and IMSI.

To estimate the depth of vacuoles, the following three treatments were performed to remove the plasma membrane and/or the acrosome from ejaculated spermatozoa from normozoospermia men: 1) induction of the acrosomal reaction, 2) freezing-thawing, and 3) demembranation with a detergent. The sperm acrosome reaction was chemically induced by the starvation method, which was originally developed for farm animals (goat, bull, and boar) (22, 23). Briefly, washed spermatozoa were resuspended in a substrate-free salt solution ($NeoK_3$; 148 mM NaCl, 2 mM $CaCl_2$, 20 mM *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, pH 7.4) at a high sperm concentration and incubated in a sealed vessel for 2–3 hours at 40°C. The acrosome reaction was evaluated by the modified triple-stain technique that combined 0.1% trypan blue vital staining (0.1% dye in HSA-HTF, for 15 minutes at 37°C), with Bismark brown counterstaining (0.5% dye in 30% ethyl alcohol, pH 2.8, for 10 minutes at 40°C) and rose Bengal acrosomal staining (0.8% dye in 0.1 M Tris buffer, pH 5.3, for 20 minutes at 24°C) (24). For freezing-thawing, washed spermatozoa were resuspended in HSA-HTF without any cryoprotectants, frozen in liquid nitrogen (−196°C), and thawed in a water bath (37°C). This freezing and thawing sequence was repeated five times. For demembranation, washed spermatozoa were permeabilized with 0.1% polyethylene glycol mono-*p*-iso-octylphenyl ether (T-8787, Triton X-100; Sigma-Aldrich) supplemented HTF for 15 minutes at room temperature.

Preparation of Epididymal and Testicular Spermatozoa and Spermatids

Epididymal and testicular spermatozoa and spermatids were obtained from azoospermic men according to the method described elsewhere (25–27). The results of preliminary biopsies indicated that spermatogenesis had been arrested at the level of the round spermatid in all nonobstructive azoospermic men. In contrast, a small number of spermatozoa were found in epididymis or testis of all obstructive azoospermic patients. After spinal anesthesia, the epididymis was exposed with a median incision of the scrotum, and spermatozoa were collected under microscopic observation by direct aspiration from the epididymal ducts using a fine glass pipette (Keisei SE-4 surgical loupes; Keisei Medical Industrial). For collection of testicular spermatozoa and spermatids, small pieces of testicular tissue were obtained through microtesticular sperm extraction (TESE). After being washed with erythrocyte-lysing buffer (28), the testicular tissues were finely minced with ophthalmic knives on ice, digested with 0.125% collagenase (Type IV-S; Sigma-Aldrich) solution containing 0.001% deoxyribonuclease (DNase) I (Sigma-Aldrich) for 20 minutes at 32.5°C with gentle agitation, washed with Dulbecco's phosphate-buffered saline (PBS) supplemented with 1% glucose and 6 mM sodium pyruvate and antibiotics (29) by centrifugation at 1,300 rpm for 3 minutes at 4°C, and finally filtered using two types (30 and 15 μm) of nylon filter sheet (NRS-030 and NRS-015; Nippon Rikagaku Kikai). Isolated spermatogenic cells were microscopically observed and spermatids were distinguished by the cytologic criteria according to Mansour et al. (30) and classified into six phases (Sa, Sb1, Sb2, Sc, Sd1, and Sd2) (31–33).

Epididymal or testicular spermatozoa and spermatids were suspended in HTF supplemented with 10% serum protein substrate (10% SPS-HTF; Sage In-Vitro Fertilization Inc., A Cooper Surgical Company) and stored at 32.5°C before observation of vacuoles, fixation for electron microscopy, and chromosomal maturation (only epididymal spermatozoa).

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